What is claimed is:

1. A method of analysing a plurality of biological entities using an imaging apparatus, the method comprising:

a) providing a marker for said plurality of biological entities, said marker being capable of identifying objects within said plurality of biological entities when detected using the imaging apparatus, the method of provision being arranged such that said marker is capable of identifying said objects during a first time period, and said marker is less capable of identifying said objects during a second time period;

b) during the first time period, recording a marked-up image in which spatial definitions of said objects are identifiable from said marker;

c) during the second time period, recording a first image of said plurality of biological entities; and

d) generating a spatial definition for an object in said first image using data derived from said marked-up image.

2. A method according to claim 1, wherein said first time period is previous to said second time period.

3. A method according to claim 1, wherein said first time period is subsequent to said second time period.

4. A method according to claim 3, comprising adding said marker to said plurality of biological entities after recording of the first image.

5. A method according to claim 2 or 3, wherein said marker has a temporally-varying signal.

6. A method according to claim 5, wherein said marker is provided by a genetic construct system.

7. A method according to any preceding claim, wherein said generated spatial definition includes at least one of a spatial extent and locational data of the object.

8. A method according to any preceding claim, wherein the generated spatial definition is generated using a spatial definition of the object detected from said marked-up image.

9. A method according to any preceding claim, the method comprising:

e) during the first time period, recording a further image of said plurality of biological entities; and

f) deriving data from said further image, and

in step d), analysing said first image using the data derived from the further image.

10. A method according to claim 9, wherein said further image is recorded in a first colour channel and said marked-up image is recorded in a second, different colour channel.

11. A method according to claim 10, wherein said first image is recorded in said first colour channel.

12. A method according to claim 9, 10 or 11, comprising, in step f), deriving data from said further image using data derived from said marked-up image.

13. A method according to any of claims 9 to 12, wherein the data derived in step f) comprises a value or values of one or more characteristics associated with the object.

14. A method according to claim 13, wherein the one or more characteristics include at least one of a mean intensity, a standard deviation, a variance, a kurtosis, an auto-correlation function, a spatial correlation measure, a textual correlation measure, an auto correlation function, a fractal dimension, an area, a perimeter, a length of a principle axis, a width of a principle axis, a compactness and an orientation.

15. A method according to any preceding claim, wherein step d) comprises:

i) defining one of a plurality of test spatial definitions;

ii) calculating a value of one or more characteristics of the first image using the test spatial definition;

iii) repeating steps i)-ii) for a different one of the plurality of test spatial definitions;

iv) selecting one of the plurality of test spatial definitions according to the value or values calculated in step ii).

16. A method according to claim 15 and any of claims 9 to 14, wherein step iv) comprises comparing said value calculated in step ii) with a value derived from said further image in step f).

17. A method according to claim 16 wherein said comparing comprises calculating a Euclidean distance E, said Euclidean distance E being calculated by the following relation:

$$E = \sqrt{\sum_{i=1}^{K} (Z_N[i] - Z_{N-1}[i])^2}$$

wherein both the value calculated in step ii) and the value derived from said further image in step f) are vectors, respectively Z_{N-1} and Z_N, relating to an integer number K of characteristics i.

18. A method according to claim 17, wherein step iv) comprises selecting a substantially minimised value of the Euclidean distance *E*.

19. A method according to claim 16, wherein said comparing comprises calculating at least one of a cityblock function, a chebyshev distance, a minkowski of order m function, a quadratic function, a Q-positive definite function, a Canberra distance, a non-near distance function, or an angular separation.

20. A method according to any preceding claim, comprising repeating step d) to generate a plurality of spatial definitions for a plurality of objects in said first image.

21. A method according to claim 20, wherein the plurality of generated spatial definitions are filtered according to a quality criterion.

22. A method according to claim 20 or 21, wherein step d) comprises determining a surrounding space of an object detected from said marked-up image, said surrounding space having a boundary separating the surrounding space from at least one different surrounding space of a proximate, different, object and arranging the generated spatial definition to be within the determined surrounding space of the object.

23. A method according to claim 22, comprising determining the surrounding space of the object using a Voronoi algorithm.

24. A method according to any preceding claim, comprising recording a second image of the plurality of biological entities during a third time period and generating a spatial definition for an object in said second image.

25. A method according to any preceding claim, wherein said biological entities are biological cells.

26. A method according to claim 25, wherein said objects comprise biological cell nuclei.

27. A method according to claim 25, wherein said objects comprise biological cell mitochondria, biological cell cytoplasm, biological cell lysosomes or bound antibodies.

28. A method according to claim 25, wherein said objects include at least two of biological cell nuclei, biological cell mitochondria, biological cell lysosomes, biological cell cytoplasm and bound antibodies.

29. A method according to claim 28, wherein when said objects include a bound antibody, said biological cells are fixed prior to said providing of the marker.

30. A method according to any preceding claim, wherein said method further comprises providing a second, different, marker for said plurality of biological entities, said second marker being additionally used to generate a spatial definition for an object in said first image.

31. A method according to claim 30, wherein said second marker is one of a biological cell dye, a biological cell mitochondria dye, a biological cell lysosome dye or a biological cell cytoplasm dye.

32. A method according to any preceding claim, further comprising analysing characteristics of the plurality of biological entities by analysing said first image using said generated spatial definition.

33. A method of image analysis for analysing a plurality of biological entities from images produced using an imaging apparatus, the method comprising:

a) obtaining a marked-up image of said plurality of biological entities, said marked-up image having been recorded during a first time period in which a marker provided for said plurality of biological entities is capable of identifying objects within said plurality of entities;

b) obtaining a first image of said plurality of biological entities, said first image having been recorded during a second time period in which said marker is less capable of identifying said objects; and

c) generating a spatial definition of an object for said first image using data derived from said marked-up image.

34. Computer software arranged to perform the method of any preceding claim.

35. A data carrier storing the computer software of claim 34.

36. Apparatus arranged to perform the method of any of claims 1-33.